Principal basis for enzyme power

Alexander G. Foigel

Institute of Theoretical and Experimental Biophysics, RAS, Pushchino 142290, Russia

E-mail: alexander_foigel@rambler.ru

The reaction rate enhancement that enzymes produce had not been fully appreciated. The object of the article is to present the mechanism of the enormous catalytic power of the enzymes. I conclude that during substrate conversion to product the enzyme transfers firstly some additional small reactant group that must be initially presented in the active site of the enzyme to bound substrate (i) ; the enzyme regenerates during second substrate group transfer (ii); the active enzyme acts as a reactant of the enzymatic reaction (iii). The detailed chemical mechanisms of enzymatic reactions, such as a well-studied reaction of the serine proteases family, peptide bond hydrolysis catalyzed by α-chymotrypsin, and the glyceraldehyde-3-phosphate interconversion step in glycolysis, are in accordance with my conclusion.

The enzymes have two main functional properties: a high degree of substrate specificity and an enormous catalytic power. Substrate specificity derives from the chemistry of the enzyme active sites and binding properties. It is widely known Fisher's "key-lock" hypothesis, in which the substrate must be adsorbed on the surface of a rigid protein in order to account for the capacity of enzymes to discriminate between several substrates. On the other hand, the mechanism of enzyme catalytic power remains unclear. The mainstream explanation of enzyme catalysis is relied on the notion that enzymes can utilize the binding energy released during association of substrates to enzymes to decrease activation energy. A widely accepted version of this concept is that the every enzyme must be complementary to the substrate transitional structure. Thus, it is assumed that the enzymatic conversion $S \xrightarrow{E} P$ of the substrates to products may be outlined by the simple general scheme

$$E + S \xrightarrow{I} ES \xrightarrow{II} EP \xrightarrow{III} E + P \qquad (1)$$

where E is the enzyme free of both reactant(s), S, and product(s), P, both ES and EP are the reaction intermediates, the numbers refer to the reaction steps.

There are a great number of theoretical works devoted to elucidation of enzyme power. However, it is not clear whether enzymatic model (1) meets the requirement of the sufficiently high rate of enzymatic reaction. Note that a factor of 10^{12} was needed to

the maximal theoretical catalytic rate of the model (1) in order to get the rate of an enzymatic reaction such as glycosidic hydrolysis[1]. Unfortunately, there are statements in some reviews that the rate accelerations that enzymes can produce are in the range of 10^8-10^{12}. At the present, the high rates of enzymatic reactions are unexplained; we can quote the emphatic saying of Wolfenden and Snider that "the rate enhancements that enzymes produce had not been fully appreciated until recently. In the absence of enzymes, these same reactions are among the slowest that have ever been measured, some with halftimes approaching the age of the Earth" [2]. The findings[3] make enzymatic reactions perfectly mysterious.

We should emphasize that the description (1) represents only a hypothesis that excludes the enzyme, for example, to be a reactant of the reaction. The main conclusion of the proposed concept of enzyme catalysis is that the active enzyme transfers to bound substrate some additional transfer group of the reaction with excess free energy. This conclusion that I had early proposed as suggestion for the case of extremely powerful hypothetical enzymes to solve the problem of above mentioned factor [4] has been supported by well-studied enzymatic reactions.

THEORETICAL RESULTS

It is unlikely that the enzyme can enhance the flux of kinetic energy of various moving particles from the medium to the substrate. In addition, the binding energy of enzyme-substrate complex cannot be considered as external free energy that is necessary for substrate activation.

Thus, I state that during conversion every enzyme involves the additional exergonic reaction that promotes substrate activation. For more detailed consideration let us start from the well established viewpoint that every reaction must be characterized by activation energy, which is equal to the heat energy, or kinetic energy, obtained by the reactants from their environment necessary to overcome the minimal potential energy barrier between the reactants and products. Therefore we may insert the transition state EX‡ complex for non-catalytic reaction (1) and use the scheme

$$\underset{}{E + S} \overset{I}{\Longrightarrow} \underset{}{ES} \overset{II}{\Longrightarrow} \underset{}{EX‡} \overset{III}{\Longrightarrow} \underset{}{EP} \overset{IY}{\Longrightarrow} \underset{}{E + P} \qquad (2)$$

where X‡ denotes the reactants in the transition state complex. It is known from transition state theory that the EX‡ complex has significant free energy excess in relation to other intermediates because of substrate bond stretching. Besides, the step III is the single source of free energy in our reaction system close to equilibrium condition due to close of whole medium to the free energy minimum (the ATP hydrolysis reaction in muscle cells under no equilibrium condition will be considered below). However, we cannot apply the EX‡ complex as an enzyme because the complex has a transient state and its working places, active site and binding place, are taken.

In accordance with our approach we have to assume the presence of some additional complex EX in our reaction medium as an active enzyme which has the following properties: specific binding place on the enzyme is free (i); there are one small transfer group X of the reaction in the active site of the enzyme (ii); this group X has free energy

excess due to more easily bound state that may be utilized after association with substrate for lowering the activation energy (iii). Then the transfer of this group to final position on the bound substrate must be considered as additional coupled reaction for subsequent substrate conversion. Besides, we must include regeneration of the active enzyme in the last step of conversion.

Thus, we have the following assumption scheme

$$\underset{}{S} + \underset{}{EX_1} \overset{I}{\Longrightarrow} \underset{}{SEX_1} \overset{II}{\Longrightarrow} \underset{}{PEX_2} \overset{III}{\Longrightarrow} P + EX_2 \qquad (3)$$

where the complicated step II includes both association of the first transfer group X_1 with bound substrate and regeneration of the enzyme by the second transfer group coming from substrate. The model (3) may be represented by general scheme

$$S \overset{EX}{\Longrightarrow} P \qquad (4)$$

where EX is the enzyme in its active form, X is the transfer group of the S ==> P reaction. I have proposed the general scheme (4) early where EX was the complex of the hypothetical enzyme with the intermediate product (Fogel, 1982). One can see that the X_1 group of the active enzyme must be appeared in the product, i. e. we represent the active enzyme as reactant of the reaction. Besides, the reaction (3) must be interpreted as the incomplete conversion of the substrate particle because its initial group X_2 must be remained on the enzyme.

In the case of cleavage of one chemical bond it is not clear how the enzyme with one active site can be regenerated during step II of the reaction (3). There are five general chemical transformations occurring in cells (oxidation-reduction, rearrangement, group transfer, cleavage, condensation) and all of them involve the breakdown of two or more bonds of the reagents. Therefore, the breakdown of the first initial bond after X_1 association with substrate and formation of the first final bond give possibility to enzyme regeneration.

CONFIRMATIONS AND CONCLUSIONS

The crucial point for verification of my concept is that the catalyst must be a complex of the enzyme with the transfer group of the reaction. This chemical aspect is in accordance with the well-known mechanisms of several families of enzymatic reactions. Let us consider the reaction of peptide bond hydrolysis catalyzed by a pure protein α-chymotrypsin (enzyme acting without cofactor), a well-studied member of the serine proteases family, see [5, 6]. I emphasize two distinguishable steps

$$S_1 + EH \Longrightarrow P_1H + EP_2 \qquad (5a)$$

$$EP_2 + \mathbf{H}\text{-}O\text{-}H \Longrightarrow E\mathbf{H} + P_2OH \qquad (5b)$$

where S_1 is a polypeptide, P_1H and P_2OH are products. The first step (5a) includes the formation of a covalent acyl-enzyme intermediate; the second step (5b) is the

deacylation step. It is important to note that the H, initially found on the enzyme, appears in the product, therefore it can be considered as an additional reaction group. The nature of it isn't clear, see below after reaction (7). Thus, the reaction (5a) shows that the enzyme acts as usual reactant of the reaction. According to proposed energetic aspect, H transport from enzyme promotes the formation of the first final chemical bond and regeneration of the enzyme in the presence of second reactant.

Let us consider another example. An important step in glycolysis is the interconversion of glyceraldehyde-3-phosphate (G3P) and dihydroxyacetone phosphate (DHAP) catalyzed by a well-studied enzyme triose phosphate isomerase. The active site of the enzyme includes a glutamate residue (Glu 165) and a histidine (His 95). The one-substrate reaction can be briefly described as the two-step conversion

$$O{=}CH\text{-}CHO\mathbf{H}\text{-}CH_2OPO_3^{2-} + EH \;\Longrightarrow\; HO\text{-}CH{=}CO\mathbf{H}\text{-}CH_2OPO_3^{2-} \ldots HE$$

G3P — Enediol intermediate

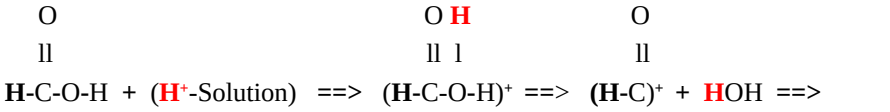
$$\Longrightarrow\; HO\text{-}CH_2\text{-}CO\text{-}CH_2OPO_3^{2-} + E\mathbf{H} \qquad (6)$$

DHAP

The first step shows the formation of an enediol intermediate that proceeds due to the transfer of His95 H group from the enzyme to the carbonyl (the reaction above outlined as additional exergonic reaction) and the transfer of C_2 proton to Glu165 of the enzyme[7, 8]. As in the former example, the H group initially found on the enzyme must be assumed as an additional reactant group. Note also that the **H** initially found on the substrate does not appear in the product, therefore reaction (6) must be considered as partial conversion of the G3P molecule. Thus, the general hypothetical scheme (1) implying complete substrate conversion to product does not be applicable for this reaction. One may see such a discrepancy in the former example as well. In addition, we stress contradiction of these two examples with widely accepted version of the enzyme catalysis conception above mentioned because we cannot find the substrate transitional structures in the examples necessary for statement of the enzyme complementarities.

Another aspect of my suggestion is that the enzyme in active form EX is a high-energy enzymatic complex that may be produced in the specific procedure. It is well known that α-chymotrypsin is initially synthesized as chymotrypsinogen that is cleaved by trypsin to expose the active enzyme site, see [9]. To obtain the active enzyme with another additional transfer group, the cofactor dependent phosphoglycerate mutase that catalyzes the transformation of 3-phosphoglycerate to 2-phosphoglycerate must be firstly phosphorylated by 2,3-bisphosphoglycerate, see [10]. The enzyme activation may be done during preincubation of the enzyme with specific agents or substrates.

Note that we arrived at the conclusion concerning the involvement of an additional reactant group without evaluation of extent of free energy transfer. Therefore this conclusion may be applied to non-enzymatic catalysis. Thus, I suggest the common principle of catalysis under close to equilibrium condition, an involvement of the additional transfer group into catalytic reaction. This hypothesis proposed by author earlier for the case of a high dielectric constant [11] may be supported by known mechanism of acid-catalyzed decomposition of formic acid:



(7)

We can see the single H^+ transfer here and there in the catalytic reaction that is impossible inside the enzyme. Therefore, the proton transfer in the enzymatic reaction above outlined is accompanied by one or two electrons transport. Electron transport must be interpreted as bond breakdown and bond formation.

The catalytic mechanism (additional free energy transfer; this requirement is necessary for catalysis in two opposite directions under equilibrium condition) does not depend on the concentration of substrates or products of the reaction in the medium. However, a shift in their concentration causes, mainly, the free energy changes of the first and final steps of the simple scheme (3) due to the changes of the free energy content of every molecule, whether S or P, in water solution. These speculations are in accord with the following results. The final step of ATP hydrolysis in skeletal muscle is the product release caused by the association of myosin heads with actin[12]. The course of events is that the closing of the actin-binding cleft during the association reaction is structurally coupled to the opening of the nucleotide-binding pocket on the myosin active site[13]. Notably, the final steps of ATP hydrolysis include the fast release of phosphate and slow ADP release[14, 15]. Release of a phosphate anion from bound ADP anion into water solution may be considered as an exergonic step. Obviously, its free energy cannot be used directly in the previous steps of hydrolysis of this ATP molecule. Besides, the excess free energy of the additional reactant cannot be utilized in muscle contraction without inhibition of ATP hydrolysis.

Thus, we arrive at the conclusion that the priority release of the phosphate leads to transformation of free energy of ATP hydrolysis into the kinetic energy of the solvated phosphate that gives an active streaming. This conclusion of such a kind of local mechanochemical transduction is consistent with the Tirosh mechanism of muscle contraction that a force in muscle is a summarized action of the active streamings from actin-bound myosin heads caused by ATP hydrolysis[16].

REFERENCES

1. Koshland, D. E. Jr. & Neet, K. E. The catalytic and regulatory properties of enzymes. *Annu. Rev. Biochem.* **37**, 359-410 (1968).
2. Wolfenden, R. & Snider, M. J. The depth of chemical time and the power of enzymes as catalysts. *Acc. Chem. Res*. **34**, 938-945 (2001).
3. Wolfenden, R. Thermodynamic and extrathermodynamic requirements of enzyme catalysis. *Biophys. Chem*. **105**, 559-572 (2003).
4. Fogel, A. G. Cooperativity of enzymatic reactions and molecular aspects of energy transduction. *Mol. Cell. Biochem*. **47**, 59-64 (1982).
5. Lehninger, A. L., Nelson, D. L. & Cox, M. M. *Principles of Biochemistry* (Worth Publishers, New York, 1993).

6. Hengge, A. C. & Stein, R. L. Role of protein conformational mobility in enzyme catalysis: acylation of α-chymotrypsin by specific peptide substrates. *Biochemistry* **43**, 742-747 (2004).
7. Davenport, R. C. *et al*. Structure of the triosephosphate isomerase-phosphoglycolohydroxamate complex: an analogue of the intermediate on the reaction pathway. *Biochemistry* **30**, 5821-5826 (1991).
8. Bash, P. A. *et al*. Computer simulation and analysis of the reaction pathway of triosephosphate isomerase. *Biochemistry* **30**, 5826-5832 (1991).
9. Wroblowski, B., Diaz, J. F., Schlitter, J. & Engelborghs, Y. Modelling pathway of alpha-chymotrypsin activation and deactivation. *Protein Engineering* **10**, 1163-1174 (1997).
10. Jedrzejas, M. J. Structure, function, and evolution of phosphoglycerate mutases: comparison with fructose-2,6-bisphosphatase, acid phosphatase, and alkaline phosphatase. *Prog. Biophys. Molec. Biol.* **73**, 263-287 (2000).
11. Foigel, A. G. The principle of cooperativity of the catalytic reactions (WINITI, Moscow, 1983).
12. Lymn, R. W. & Taylor, E. W. Mechanism of adenosine triphosphate hydrolysis by actomyosin. *Biochemistry* **10**, 4617-4624 (1971).
13. Holmes, K. C., Angert, I., Kull, F. J., Jahn, W. & Schröder, R. R. Electron cryo-microscopy shows how strong binding of myosin to actin releases nucleotide. *Nature* **425**, 423-427 (2003).
14. Siemankowski, R. F.; Wiseman, M. O. & White, H. D. ADP dissociation from actomyosin subfragment 1 is sufficiently slow to limit the unloaded shortening velocity in vertebrate muscle. *Proc. Natl. Acad. Sci. USA* **82**, 658-662 (1985).
15. White, H. D., Belknap, B. & Webb, M. R. Kinetics of nucleoside triphosphate cleavage and phosphate release steps by associated rabbit skeletal actomyosin, measured using a novel fluorescent probe for phosphate. *Biochemistry* **36**, 11828-11836 (1997).
16. Tirosh, R., Low, W. Z. & Oplatka, A. Translational motion of actin filaments in the presence of heavy meromyosin and MgATP as measured by Doppler broadening of laser light scattering. *Biochim. Biophys. Acta* **1037**, 274-280 (1990).

ACKNOWLEGEMENTS

I thank Prof. E. I. Maevsky and Prof. Y. G. Kaminsky for remarks to my paper and R. Tirosh of Israel Bar-Ilan University for detailed explanation of his muscle contraction mechanism at submolecular level.